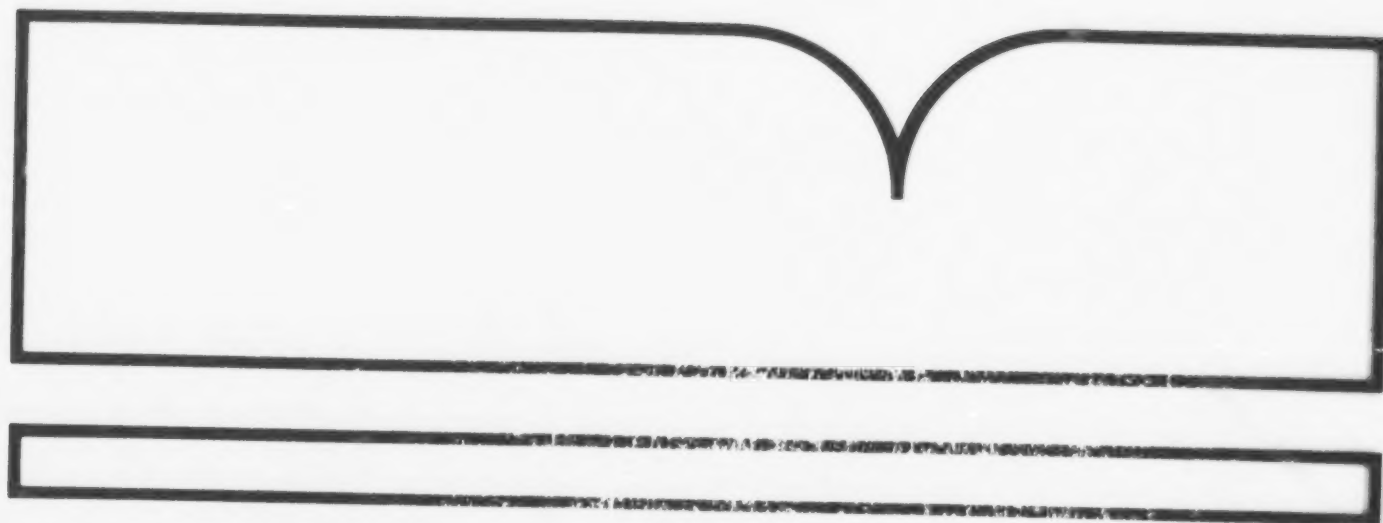


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Biochemical Effects of Sulfuric Acid Mist
Inhalation by Human Subjects while at Rest

(U.S.) Health Effects Research Lab.
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Biochemical Effects of Sulfuric Acid Mist Inhalation by Human Subjects while at Rest

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ABSTRACT. This study evaluated the effect of sulfuric acid aerosol exposure for 2 consecutive days on seven human biochemical blood parameters. A total of 20 human subjects were exposed to $100 \mu\text{g}/\text{m}^3$ ($0.033 \mu\text{M}$) sulfuric acid aerosol ($0.5 \mu\text{m}$ mean mass diameter) for 4 hr/day for 2 consecutive days. A total of 17 human subjects were exposed to 4 hr of ambient air on both exposure days. The chemical blood parameters were measured pre- and post-exposure, and 20 hr after the second exposure: serum glutathione, red blood cell glutathione reductase, red blood cell glucose-6-phosphate dehydrogenase, lysozyme, serum glutamic oxaloacetic acid transaminase, serum vitamin E, and 2, 3-diphosphoglycerate. The results indicate no significant response in any of the seven biochemical blood parameters measured. At this level, repeated exposure did not over-burden the upper airway defenses against acid aerosol.

WITH THE INCREASE of automobile catalytic converters and the use of high sulfur content coal as an energy source, the peak levels of sulfuric acid (H_2SO_4) could fore-

seeably rise by as much as three to four times the present level. Thus, it becomes necessary to identify and measure the possible health effects of H_2SO_4 aerosol in humans.

Evidence for any aggravation and/or causation of changes in human health induced by H_2SO_4 aerosol has been sparse. The majority of the biochemical parameters measured after H_2SO_4 aerosol exposure have been in animal models. Early animal H_2SO_4 exposure studies indicated there is a hierarchy of toxicity (guinea pigs > mice > rats > rabbits).¹ In addition, it was reported that the toxicity within a species is age dependent.² Lewis et al.³ measured hematological changes and pulmonary function in female beagles exposed to $13.4 \text{ mg}/\text{m}^3$ sulfur dioxide (SO_2) and $0.9 \text{ mg}/\text{m}^3$ H_2SO_4 alone and in combination. They concluded that: (a) chronic exposure to relatively low levels of H_2SO_4 can produce serious pulmonary impairment; (b) lungs previously exposed to a toxic agent, nitrogen dioxide (NO_2) in their study, are less likely to show altered lung physiology upon subsequent exposure to low levels of airborne pollutants; and (c) no significant changes in hematology occurred as a result of the exposure.

Alarie et al.⁴ reported that long-term exposures of cynomolgus monkeys and guinea pigs to H_2SO_4 mist at

concentrations varying from 0.38 to 4.79 mg/m³ produced no changes in hematological parameters, while the routine biochemical screening studies showed considerable variation but no discernible trends.

Human exposure studies reported in the literature have concentrated on pulmonary function effects. Amdur et al.¹³ reported rapid and shallow respiration in normal men exposed to 350 to 5000 µg/m³ H₂SO₄. Increased respiration rate results in decreased retention of inhaled particles. It would then appear possible that the more rapid, shallower breathing that was observed is a reflex protective mechanism to prevent retention of the acid mist.

Sim and Pattle¹⁴ studied the response of healthy males aged 18 to 46 yr to H₂SO₄ mist. Subjects were either exposed to H₂SO₄ mist (4.1–39 mg/m³) for 10 min by mask or exposed to H₂SO₄ mist (2.9–39 mg/m³) for 60 min in an exposure chamber. They measured the irritant effect of H₂SO₄ mist at a relative humidity of 62% or 90%, and found H₂SO₄ to be more irritating under conditions of high humidity. No airway resistance measurements could be made during the first 10 min. When the coughing, which was continuous throughout the exposure periods diminished enough to permit measurement, the increases ranged from 43% to 150% above normal.

This study was undertaken to evaluate the effects of 0.1 mg/m³ H₂SO₄ aerosol on seven human biochemical blood parameters. The 0.1 mg/m³ H₂SO₄ aerosol is approximately 75% less than the concentration used in the above studies, and five to six times the level found in ambient air.

METHODS AND MATERIALS

Subjects. A total of 37 healthy, nonsmoking, Caucasian male university students characterized by an average height of 180.6 cm [standard deviation (SD), 5.1 cm; range, 168.0 to 194.0 cm], an average weight of 73.0 kg (SD, 8.0 kg; range, 56.5 to 89.5 kg), and an average age of 22.4 yr (SD, 3.2 yr; range, 18.4 to 29.7 yr), were used in this study. A total of 17 subjects were exposed to air (Group 1, controls), and a total of 20 subjects were exposed to 100 µg/m³ H₂SO₄ aerosol (Group 2, experimental). All participants were informed of any risks involved in the study. Each participant signed an informed consent statement with the understanding that withdrawal at any time during the study would be allowed.

Procedure. Each subject was exposed at rest for 4 hr on 2 consecutive days. On each day, blood was drawn immediately preceding and following the 4-hr exposure. A 20-hr post-exposure blood sample was drawn the morning after the second exposure day. Subjects were tested in groups of three.

Experimental design. The control group of subjects was exposed to ambient air on the first and second days of exposure. The experimental group was exposed to 2 consecutive days of 100 µg/m³ H₂SO₄ aerosol [0.5 µm mean mass diameter (MMD)]. The exposure began and ended at the same times each day. Data were collected in a counter-balanced fashion. During the first week, a group of three acid-exposure subjects was tested on Monday and Tuesday and a group of three air-exposure subjects were tested on Wednesday and Thursday. The following week the order

was reversed. This alternation scheme was continued throughout the experiment, and helped control any day-of-week effects and time-of-year effects.

The chamber atmosphere was maintained at a temperature of 20°C, a relative humidity of 40%, and an air flow of 227 m³ min.

A total of seven serum and red blood cell (RBC) biochemical measurements were chosen as dependent variables: serum glutathione (GSH), RBC glutathione reductase, RBC glucose-6-phosphate dehydrogenase (G6PDH), lysozyme, serum glutamic oxaloacetic acid transaminase (SGOT), serum vitamin E, and 2,3-diphosphoglycerate (2,3-DPG). Measurements were taken on all seven variables preceding and following both exposure days, and 20 hr after the second exposure day.

Multivariate analysis of variance (MANOVA) was used to analyze the results. Each of the seven blood parameters were measured five times to give 35 dependent variables. Three a priori contrasts were chosen to assess the initial difference between the air and acid groups, a time effect for air only, and an acid effect, i.e., the group by time interaction.

Blood analysis. Serum glutathione was measured by the method of Patterson and Lazarow.^{5,6} Red blood cell glutathione reductase was assayed by the method of Nicholls,⁷ modified such that the enzyme was preincubated at 0°C for 30 min in the presence of 60 µM FAD and the reaction was initiated by the simultaneous addition of 2 mM oxidized glutathione (GSSG) and 0.24 mM NADPH. Red blood cell G6PDH was assayed by the procedure of Catalano,⁸ lysozyme by the method of Shugar,⁹ serum vitamin E by the method of Chaney (unpublished procedure), 2,3-DPG in erythrocytes by the Nygaard and Rorth¹⁰ method, and SGOT was analyzed on the Centrifichem 400 autoanalyzer by a modified Karmen technique.^{11,12}

A Centrifichem Model 400 centrifugal analyzer and Centrifichem autopipetter (Union Carbide Corporation, Clinical Diagnostics Division, Rye, NY 10580) were used for assaying SGOT and G6PDH. Manual assays for glutathione reductase, GSH, and 2,3-DPG were run on a Cary 118 spectrophotometer. The lysozyme assay was run on a Varian 635 spectrophotometer.

SGOT reagent kits were purchased from Union Carbide Corporation (Clinical Diagnostics Division, Rye, NY 10580); 2,3-DPG reagent kits were purchased from Calbiochem (La Jolla, CA 92037); and lysozyme reagent kits were purchased from Worthington Diagnostics (Freehold, NJ 07728).

D, L-α-tocopherol, GSSH (free acid, grade III), FAD (disodium salt, grade III), NADPH (tetrasodium salt, type 1), NADP⁺ (monosodium salt, Sigma Grade), 6-phosphogluconic dehydrogenase (Type V from yeast, 50 U/ml), D-glucose-6-phosphate monosodium salt, alloxan monohydrate, and GSH (reduced form, 98–100% purity), were purchased from Sigma Chemical Company (St. Louis, MO 63178).

RESULTS

Table 1 provides summary statistics for all seven dependent variables for the initial blood draw. Data from

Table 1.—Summary of Statistics for All Seven Dependent Variables for Pre-Day 1*

Variable	Mean	SD	Min.	Max.	Skewness
GSH (mg/100 ml)	38.45	4.80	29.46	47.97	0.10
GSH reductase (μ mole/gm Hgb/min)†	7.45	3.19	1.77	12.95	-0.28
RBC G6PDH (U/gm Hgb)	2.04	0.26	1.54	2.77	0.47
Lysozyme (μ g/ml)	9.67	2.04	5.41	14.78	0.64
SGOT (IU/L)	11.7	2.5	6.5	17.5	0.51
Vitamin E (μ g/ml)	5.22	1.51	2.70	7.78	0.69
2,3-DPG (μ mole/ml RBC)	4.61	0.92	3.04	7.81	1.14

*N = 37

†Hgb = hemoglobin

all 37 subjects has been combined since none of the subjects had been exposed to any treatment at the time of this initial blood draw. The means, standard deviations, ranges, and skewnesses are reasonable for the purposes of the MANOVA.

Table 2 summarizes the MANOVA for the seven dependent variables. All tests presented are multivariate tests, i.e., all seven variables are treated simultaneously. The three contrasts computed require explanation. The first contrast is labeled group difference at pre-1. This tests the hypothesis of no difference on any of the variables for the treatment and control groups (H_2SO_4 group and air group). Since this test is not significant ($P = .20$), the hypothesis that the two groups began the experiment with the same values for the variables measured may not be rejected.

The second contrast tested evaluates the presence of any difference across time for the air exposure group by itself. This is, therefore, the simple effect of time within air. Since the multivariate test is not significant ($P = .51$), the hypothesis that the mean levels are equal for all time points cannot be rejected.

The third contrast tests for any effect of H_2SO_4 exposure. This is the group by time interaction, which was determined insignificant ($P = .47$), thus indicating no acid

effect on any variable.

Table 3 provides mean response on all seven dependent variables for each group at each time point. These same means are presented graphically in Figures 1 through 7. These results indicate only small variations across time and groups. The standard deviations in Table 1 are representative of the standard deviations associated with the other time points.

The results indicate no response observed in any of the seven biochemical blood parameters measured as a result of the two 4-hr exposures to $100 \mu g/m^3$ ($0.033 \mu M$) H_2SO_4 aerosol (0.5 MMD) on 2 consecutive days. Similar results were obtained in a study in which human subjects were

Table 3.—Mean Response on All Seven Dependent Variables, Estimated from MANOVA

Variable	Treatment	Day 1		Day 2		Day 3
		Pre	Post	Pre	Post	Pre
Glutathione (mg/100 ml)	Air-air	38.60	39.82	40.25	40.68	39.03
	Acid-acid	38.32	39.52	39.41	42.00	38.61
Glutathione reductase (μ mole/gm Hgb/min)	Air-air	8.22	8.15	8.41	7.82	7.83
	Acid-acid	6.80	7.33	7.08	7.41	7.16
RBC G6PDH (U/gm Hgb)	Air-air	2.06	2.05	2.08	2.09	2.20
	Acid-acid	2.03	2.01	2.01	2.09	2.15
Lysozyme (μ g/ml)	Air-air	10.56	10.09	10.28	10.26	10.68
	Acid-acid	8.91	8.35	8.96	9.01	9.03
SGOT (IU/L)	Air-air	11.3	11.2	12.0	11.5	11.8
	Acid-acid	11.9	11.8	11.5	11.4	11.7
Vitamin E (μ g/ml)	Air-air	4.89	4.84	5.13	5.15	5.12
	Acid-acid	5.49	5.03	4.97	5.34	4.87
2,3-DPG (μ mole/ml RBC)	Air-air	4.86	4.97	4.75	4.68	5.02
	Acid-acid	4.40	4.50	4.49	4.61	3.86

exposed to one 4-hr exposure to $100 \mu g/m^3$ H_2SO_4 aerosol (unpublished data).

There are two possible ways that nonsignificant results can arise: if there is great variability due to lack of precision, all but very large effects will be masked; and conversely, with good precision, a nonsignificant result is indicated by very small differences among groups. In the

Table 2.—MANOVA Summary for All Seven Dependent Variables

Source	Likelihood Ratio	$\tilde{F}^*$	Numerator df	Denominator df	P
Group difference at pre day 1 exposure	.784	1.51	6	33	.20
Air group time effect	.384	1.00	24	15	.51
Acid effect (group X time)	.371	1.06	24	15	.47
*Rao's F approximation					

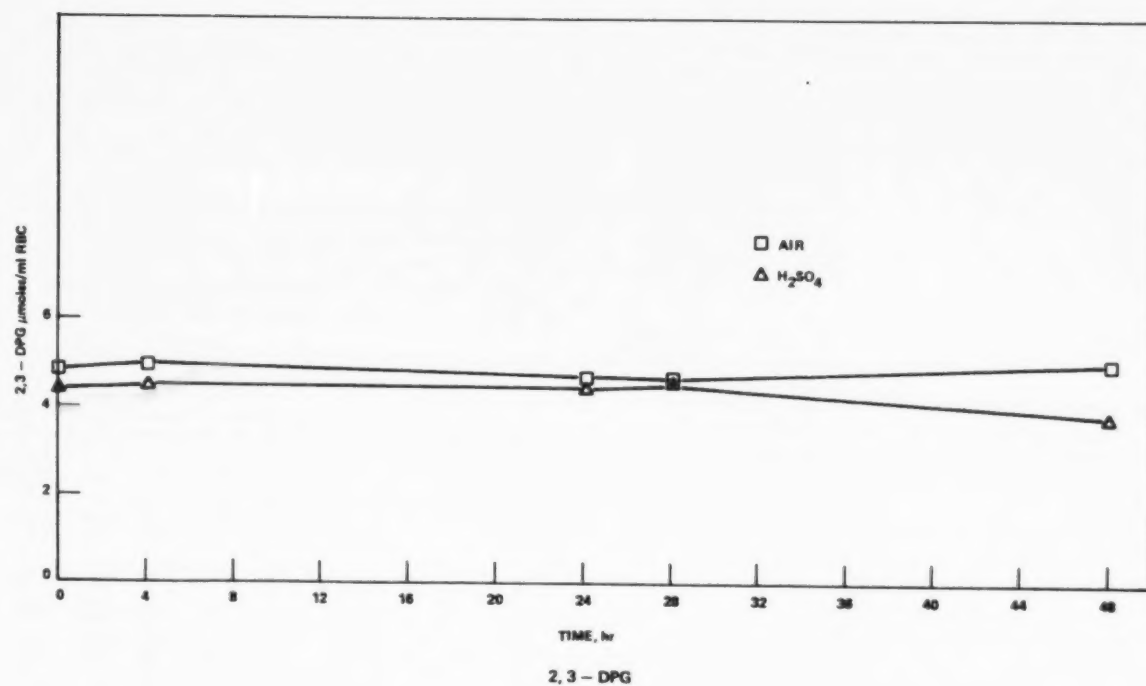


Fig. 1. Acid by time means for 2,3-DPG.

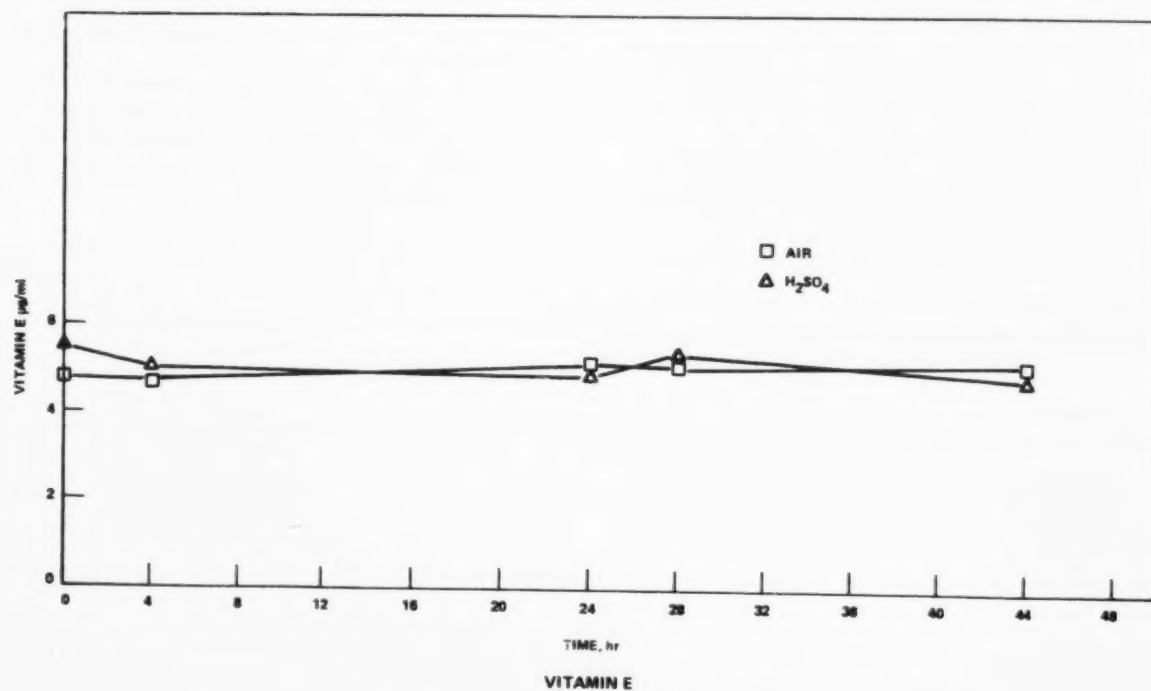


Fig. 2. Acid by time means for serum vitamin E.

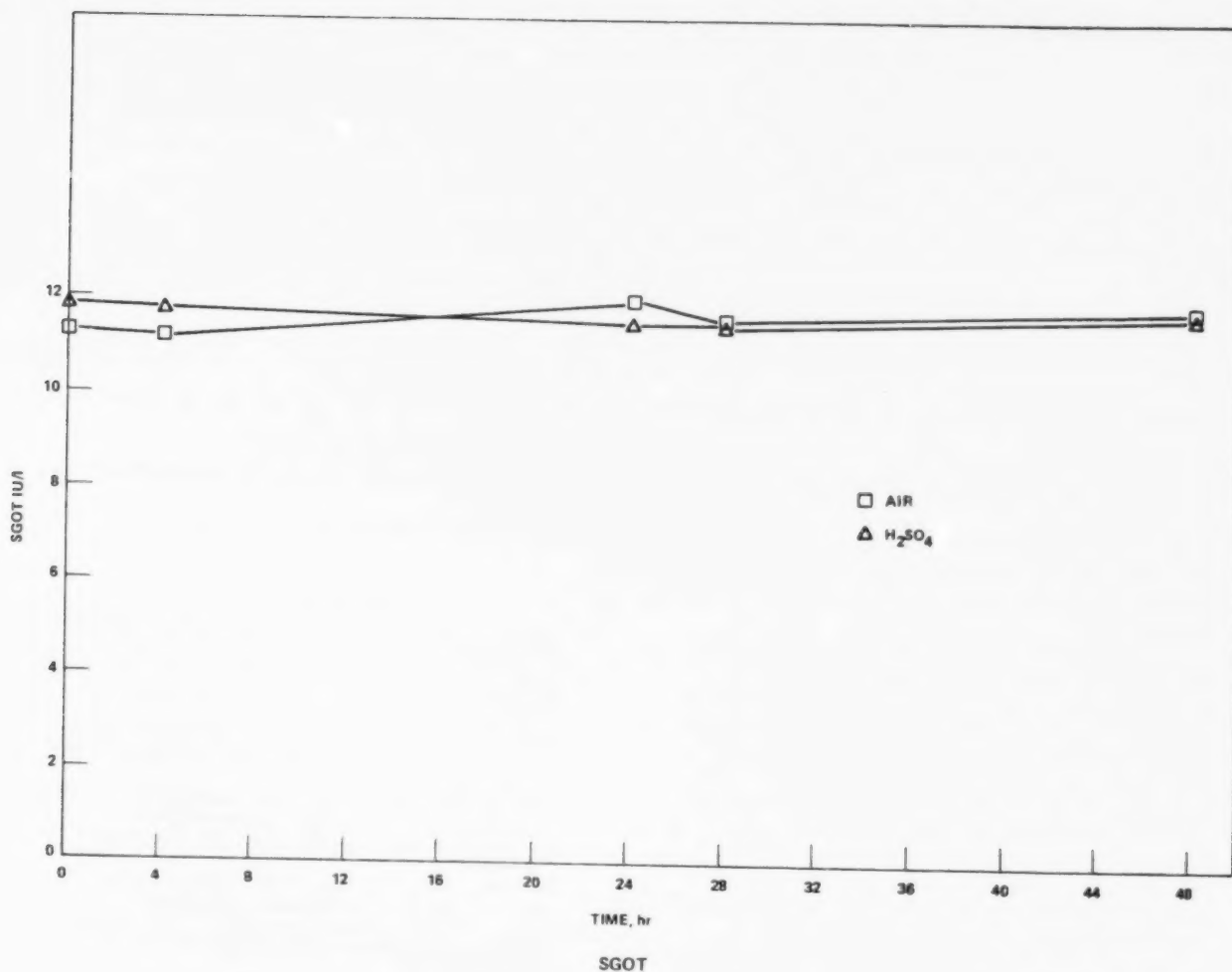


Fig. 3. Acid by time means for SGOT.

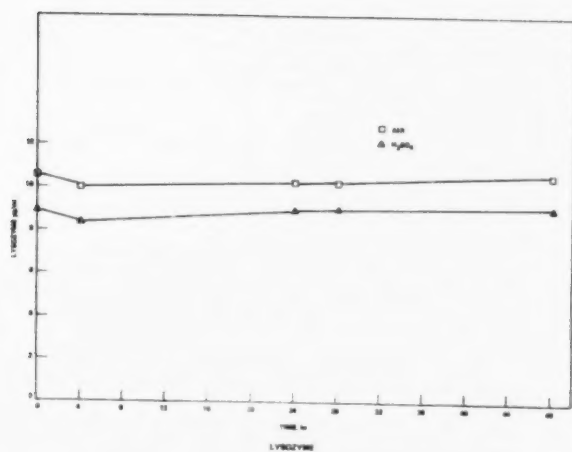


Fig. 4. Acid by time means for lysozyme.

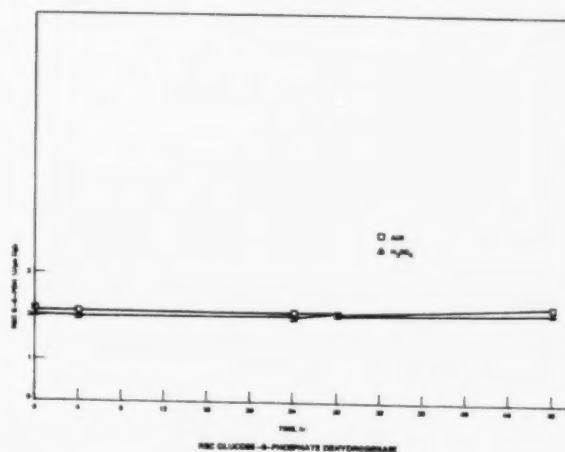


Fig. 5. Acid by time means for RBC G6PDH.

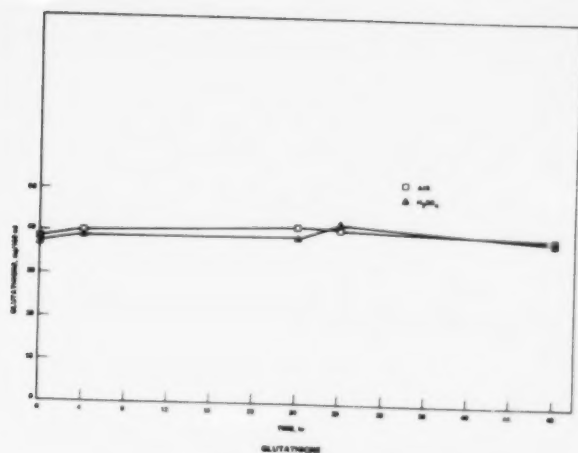


Fig. 6. Acid by time means for GSH.

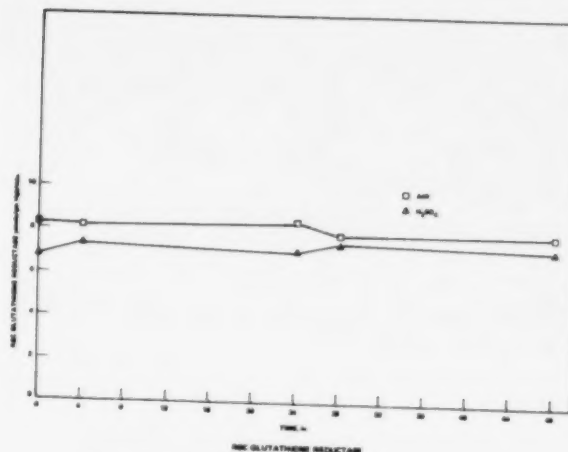


Fig. 7. Acid by time means for RBC GSH reductase.

latter case, if any effects do exist, they are very small. In the former case, one may only report that no effects were detectable. The authors feel that these data conform to the second pattern.

The results reported are consistent with the available animal studies. Even long-term continuous exposure of monkeys to H_2SO_4 mist at concentrations varying from 380 to 4790 $\mu\text{g}/\text{m}^3$ (submicronic particles to 4 μ MMD) and guinea pigs to concentrations at 80 and 100 $\mu\text{g}/\text{m}^3$ with MMD of 0.8 μm , respectively, resulted in no trend, indicating possible effects from H_2SO_4 on hematologic and serum biochemical parameters.⁴

Earlier human exposure studies reported pulmonary function effects only. The authors are not aware of any studies on the response of human blood parameters upon exposure to H_2SO_4 mist.

In reality, the effects of H_2SO_4 mist may not go beyond the upper respiratory tract. The ammonia released by the respiratory system may well eliminate any effect of inhaled H_2SO_4 aerosol through partial or complete neutralization. The findings of Pattle et al.¹⁵ support this hypothesis. While measuring the 8-hr lethal concentration 50% probable for guinea pigs exposed to H_2SO_4 , it was found that ammonium carbonate present in the chamber in an amount sufficient to provide an excess of ammonia gave protection from levels of H_2SO_4 , which in the absence of ammonia, would have caused 50% mortality.

In conclusion, no significant effect of H_2SO_4 mist was found for any of the blood parameters measured. This finding is consistent with previous animal studies on biochemical blood parameters. Earlier studies involving humans have indicated pulmonary function effects, however, no previous results appear to have been published on human biochemical blood parameters. It is possible that, at the level of H_2SO_4 studied, the body's defense mechanisms limit any effects to the upper airways.

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